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EFFECT OF LOCAL MEDICINAL PLANTS ON GROWTH OF LEAF SPOT DISEASE – CAUSING *Colletotrichum gloeosporiodes*

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Abstract

Local medicinal plant extracts for controlling clinical *Colletotrichum gloeosporiodes* growth were *in vitro* evaluated. This pathogen was initially isolated from pustule formation on leaves of the rubber tree generally planted in Suwari Sub-district, Resoh District, Narathiwat Province, Thailand. Microscopically, the isolated *C. gloeosporioides* showed well-branching and septated mycelia. Colonies on Potato Dextrose agar (PDA) ranged from colorless to dark brown appearance and with conidia-originated fruiting; the so-called acervulus. As for inhibition effect of medicinal plant extracts, the ethanol- and water-extracted; Galanga (*Alpinia galanga* (L.) Swartz.), Wildbetal Leafbush (*Piper sarmentosum* Roxb.), Ringworm Bush (*Cassia alata* (L.) Roxb.), Lemongrass (*Cymbopogon citratus* Stapf.), and Heart-Leaved Moonseed (*Tinospora crispa* (L.) Miers ex Hook.f. & Thomson), were tested using Poisoned Food Technique. It was found that PDA with extracts at the concentrations of 5, 1.2, 0.3, 0.2 and 0.1 µg/ml showed different inhibiting activity and that 95% of ethanol extract of *Piper sarmentosum* Roxb. gave highest (100%) inhibiting at 0.2 µg/ml. Next was of *Cymbopogon citratus* Stapf. at 5 µg/ml. Ethanol extraction in comparison with water was better solvent for the extraction. These primary findings were of potential in further development and application.

Keywords: *Colletotrichum gloeosporiodes*, Medicinal plant extracts, Leaf spot disease.

INTRODUCTION

Colletotrichum gloeosporiodes causal microorganism of leaf spot disease in leaves of rubber tree, mostly at the leaf tip region. Spots are small, brown in colour and is sorrounded by an yellow hallo. Numerous spots coalesce and dry up leading to defoliation. The infected leaves often crinkle and become distorted before shedding. *C. gloeosporioides* causing both anthracnose and papery lesions. This is the first record of *Colletotrichum* sp. on *Hevea* in India. (Saha et. al., 2002) *C. gloeosporiodes* known to infect a variety of hosts with characteristic symptoms. There is a great variation in the symptoms produced by *C. gloeosporioides* on host plants. The symptoms may be regular to irregular, round to oval, watersoaked, and sunken spots. Similarly, the fungal characteristic on culture media also varies with the host. The growing fungal colonies on culture media may be circular, wooly or cottony with characteristic colour. Vegetative hyphae observed were hyaline, simple, septate and branched. Conidia are straight, oblong or cylindrical with rounded or bulbous ends, hyaline, aseptate, one celled and dumbbell shaped. Setae are brown. (Ajay ,2014).Galanga (*Alpinia galanga* (L.) Swartz.), Wildbetal Leafbush (*Piper sarmentosum* Roxb.), Ringworm Bush (*Cassia alata* (L.) Roxb.), Lemongrass (*Cymbopogon citratus* Stapf.), and Heart-Leaved Moonseed (*Tinospora crispa* (L.)

Miers ex Hook.f. & Thomson) are ethnomedicinal plants with long application in Thailand and other Asian countries.

Galanga (*A. galanga* (L.) Swartz.), has long being used in medication, culinary and cosmetics, widely used in *dietary* intake as well as in the traditional system of medicine of Chinese and Thai folk medicine (Yang, 1999). For its rhizome of characteristic fragmance and pungency, it is widely used as and suitable for condiment for foods and local medicine in China and Thailand (Farnsworth, 1992). Wildbetal Leafbush (*P. sarmentosum* Roxb.) is traditionally used its leaves as condiment in many South Asian cuisines, and ethnomedicinally consumed for their carminative, anti-inflammatory, expectorant and anodyne properties (Jansen, 1999). Ringworm Bush (*C. alata* (L.) Roxb.) has been reported to have various phytochemical and biological activities, and used for antimicrobial and antifungal.purposes (Somchit, 2003). Lemongrass (*C. citratus* Stapf.) is also widely used in traditional medicine in Cuba and in many other countries of the Caribbean region due to its attributable popular properties of analgesic and anti-inflammatory actions (Ortiz et al., 2002). With major alkaloids composition as protoberberine type, and N-acylaporphine alkaloids are non-quaternary alkaloids, Heart-Leaved Moonseed (*T. crispa* (L.) Miers ex Hook.f. & Thomson) is widely and effectively used as Malay and Thai ethnomedicine for the treatment of hypertension and diabetes (Dweck & Cavin, 2015). In the current study, evaluation of anti-fungal property was preliminarily evaluated against clinically isolated plant pathogenic fungi from leaf spot rubber tree in persuing alternative biocontrol of plant pathogens for potential replacement of wide use of pesticides, which implicated with environmental issues.

RESEARCH METHODS

Isolation of *C. gloeosporioides* from rubber leaves. The leaves samples collected from Resoh District, Narathiwart Province, Thailand were subjected for pathogenic fungal isolation using only the small sized cut leaf pieces from lesion sites. These leaf pieces were then surface-sterilized using 10% chlorox and sterile distilled, and partially dried using sterile filter paper prior to cultivation on agar Potato Dextrose Agar (PDA, Difco, USA) supplemented with mixture 0.5 ml of 25% lactic acid. Following incubation at 25-30 °C for 5-7 days, fungal colonies emerged was morphologically and microscopically characterized, and *C. gloeosporioides* were purified and periodically maintained in PDA slant at 4°C until further investigation.

Extraction of local medicinal plant. Medicinal plant ethanol extraction was conducted based on established protocol described elsewhere. Plant parts were washed, dried, cut into pieces, and ground into powder, and later soaked in 95% ethanol for 48 hours. Extracted solution was subsequently filtered and evaporated using Rotavapor and harvested as dried extract for using in further test. Plant water extraction was conducted in similar to ethanol extraction protocol.

Antifungal assay. The antifungal assay of each ethnomedicinal plant extract was carried out against clinical isolated *C. gloeosporioides* using Poisoned Food Technique as suggested by Alam (2004) with a slight modification. In brief, PDAs modified by mixing with each extract to final concentrations of 5, 1.2, 0.3, 0.2 and 0.1 µg/ml were prepared, and subsequently a fully growth *C. gloeosporioides* mycelial bored cut was inoculated at the centre of the medium. After incubation at 25°C for 5 days, colony size appeared was measured, and minimum concentration of inhibition (MIC) was determinded using method as proposed by (Sandar et al, 1995):

$$\% \text{ Inhibition} = \frac{X - Y}{X} \times 100$$

Whereas X: diameter of fungal colony grown on control plate
Y: diameter of fungal colony grown on plates containing crude plants extracts.

RESULT AND DISCUSSION

Macroscopical and microscopical characteristics of isolated *C. gloeosporiodes*.

C. gloeosporioides colonies on Potato Dextrose agar (PDA) ranged from colorless to dark brown appearance, wooly or cottony with characteristic color. Miicroscopically, the fungi showed well-branching and septated mycelia. Vegetative hyphae observed were hyaline, simple, septate and branched. Conidia are straight, oblong or cylindrical with rounded or bulbous ends, hyaline, aseptate, one celled and dumbbell shaped. Setae are brown. (Figure 1).

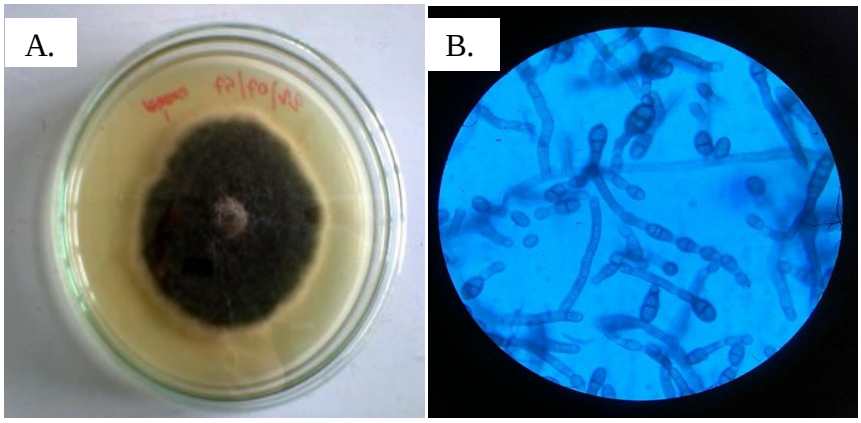

Figure1: (A).Characteristics of *C. gloeosporioides* on Potato Dextrose Agar (PDA) incubated at room temperature within 7 days . (B). Microscopic micrograph of *C. gloeosporioides* (400x)

Effect of ethanol plant extracts. All ethanol crude extracts showed inhibition against clinical isolates of *C. gloeosporioides*, with highest percentage of inhibition (100%) shown by of *P. sarmentosum* and *Cymbopogon citrates* Stapf. at concentration as low as 0.2 and 5 µg/mL. Whereas, other crude extracts of *A. galanga* (L), *T. crispa* (L.) Miers ex Hook.f. & Thomson and *C. alata* (L.) Roxb gave varying percentages of 79.78, 39.89 and 36.76, respectively. Furthermore, it was observed that *difference* crude extracts effected *C. gloeosporioides* growth at their concentration at minimum ranging from 0.1 µg/ ml (*P. sarmentosum*, *C. citrates* Stapf. and *A. galangal* (L.)) to 0.3 µg/ ml (*T. crispa* (L.) Miers ex Hook.f. & Thomson and *C. alata* (L.) Roxb.). As for minimal concentration of inhibition (MIC) of ethanol crude extract it was observed that their values were different ranging from 0.1 µg/ml or below to 0.3 µg/ml. Detailed results were shown in Table 1 and Figure 2.

Crude extracts from ethnomedicinal plants effective against *C. gloeosporioides* from different origins were reported by many workers with varying degree of results. Bussaman et al. (2012) reported methanol extract of *P. sarmentosum* inhibited 100% growth of mango-infected *C. gloeosporioides*. Therefore, result in this study about medicinal plants which can against highest of *C. gloeosporioides* as *P. sarmentosum*. It

was support this studies. While, chloroform crude *A. galanga* leaf extract exhibited the highest antifungal activities (63.69% at 10.00 µg/ml) against *C. gloeosporioides* (Johnny et al., 2010). *C. alata* (L.) Roxb. was reported to inhibit rubber tree-infected *C. gloeosporioides* by 9.90% when tested against crude extract at varying percentage (Ogbebor et al., 2007)

Table 1: Inhibition effect of ethanol crude plant extracts on *C. gloeosporioides* growth. Each value represents with mean ± standard error

Medicinal Plant	**Percentage inhibition of *C. gloeosporioides* (%)**					
	control	**0.1 µg/ml**	**0.2 µg/ml**	**0.3 µg/ml**	**1.2 µg/ml**	**5 µg/ml**
P. sarmentosum	0.00	24.24 ±0.02	100±0.00	100±0.00	100±0.00	100±0.00
C. citrates Stapf.	0.00	27.14±0.02	30.30±0.62	42.72±1.02	60.20±0.72	100±0.00
A. galangal (L.)	0.00	21.21±0.02	49.97±1.02	54.55 ± 0.92	57.71± 1.20	79.78±1.20
T. crispa (L.) Miers ex Hook.f. & Thomson	0.00	0.00±0.00	0.00±0.00	11.45±0.02	30.80±0.02	39.89±1.15
C. alata (L.) Roxb.	0.00	0.00±0.00	0.00±0.00	10.68±1.20	28.30±0.12	36.76±0.47

Figure 2. Effect of different ethnomedicinal concentration on *C. gloeosporoides* growth tested using modified PDA. A, referred to PDA modified by *P. sarmentosum*, B, *C. citratus* Stapf., C, *A. galanga* L., D, *T. crispa* (L.) Miers ex Hook.f. & Thomson and E, *C. alata* (L.) Roxb.

Effect of water plant extracts. Inhibition of mycelial growth of *Colletotrichum gloeosporioides*: All water crude extracts showed inhibition against clinical isolates of *C. gloeosporioides*, with highest percentage of inhibition (57.57%) shown by of *P. sarmentosum* at concentration 5 µg/ml. Whereas, other crude extracts of *C. citratus* Stapf., *A. galanga* (L), *T. crispa* (L.) Miers ex Hook.f. & Thomson and *C. alata* (L.) Roxb gave varying percentages of 24.24, 15.76, 20.30 and 20.24 respectively. Furthermore, it was observed that difference crude extracts effected *C. gloeosporioides* growth at their concentration at minimum 0.1 µg/ ml (*P. sarmentosum, C.citrates* Stapf., *A. galangal* (L.) and *Tinospora crispa* (L.) Miers ex Hook.f. & Thomson) to 1.2 µg/ ml (*Cassia alata* (L.) Roxb.). As for minimal concentration of inhibition (MIC) of water crude extract it was observed that their values were different ranging from 0.1 µg/ml or below to 1.2 µg/ml. Detailed results were shown in Table 2 and Figure 3.
C. gloeosporioides clinically isolated from coffee plant were found sensitive to 20% water crude extract of *Cymbopogon citrates* Stapf. (Silva, 2014).

Table 2: Inhibition effect of water crude plant extracts on *C. gloeosporioides* growth. Each value represents with mean ± standard error

Medicinal Plant	**Percentage inhibition of *C. gloeosporioides* (%)**					
	control	**0.1 µg/ml**	**0.2 µg/ml**	**0.3 µg/ml**	**1.2 µg/ml**	**5 µg/ml**
P. sarmentosum	0.00	24.24±0.02	46.36±1.3	50.39±0.2	52.51±0.6	57.57±0.8
C. citratus Stapf.	0.00	12.32±0.02	18.48±0.02	21.91±0.1	23.64±0.2	24.24±0.4
A. galangal L.	0.00	08.03±0.02	12.06±0.02	12.34±0.05	14.54±0.05	15.76±0.05
T. crispa (L.) Miers ex Hook.f. & Thomson	0.00	09.11±0.15	09.27±0.02	11.21±0.04	17.27±0.1	20.30±0.3
C. alata (L.) Roxb.	0.00	0±0.00	0±0.00	0±0.00	10.56±0.1	20.24±0.2

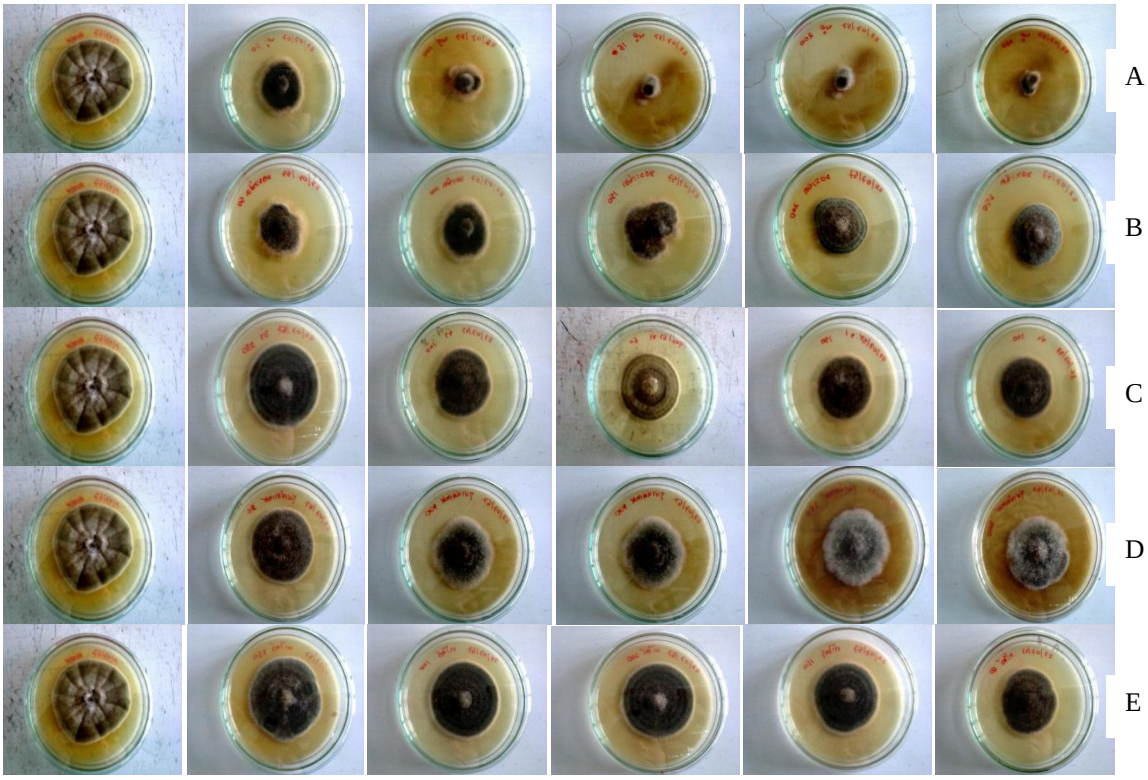

Figure 3: Effect of different water crude plant extracts concentration on *C. gloeosporoides* growth tested using modified PDA. A, referred to PDA modified by *P. sarmentosum*, B, *C. citratus* Stapf., C, *A. galanga* L., D, *T. crispa* (L.) Miers ex Hook.f. & Thomson and E, *C. alata* (L.) Roxb.

CONCLUSION AND SUGGESTION

Ethanol plant extracts of *P. sarmentosum* showed 100% inhibition activity against rubber tree-infected *C. gloeosporoides* with minimal inhibition concentration at 0.2 µg/ml. Others at 5 µg/ml concentration were *C. citratus* Stapf., *A. galanga* L., *T. crispa* (L.) Miers ex Hook.f. &Thomson (D) and *C. alata* (L.) Roxb. with inhibition percentages of 100, 79.78, 39.89 and 36.76, respectively. Minimum inhibition of concentration (MIC) of different crude extracts tested against rubber tree-infected *C. gloeosporoides* ranged from less than 0.1 to 0.3 µg/ml. This preliminary result was believed to have potential start in persuing alternative biocontrol of plant pathogens for replacement of wide use of pesticides, which implicated with environmental issues.

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